MODELING OF FORCE-VOLUME IMAGES IN ATOMIC FORCE MICROSCOPY

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ABSTRACT

Atomic force microscopy (AFM) is a recent technique generating tridimensional images at nanometric scale whatever the nature of the chemical sample. An AFM microscope affords the measurement of interatomic forces exerting between a probe associated to a cantilever and a chemical sample. A force spectrum $f(z)$ shows the force evolution as a function of the probe-sample distance z. A reproduction of this analysis in conjunction with the scan of the sample surface yields a force-volume image $f(x, y, z)$. Today, the analysis of a force-volume image remains mainly descriptive. We introduce a signal processing formulation aiming at a precise characterization of each pixel (x, y) of the sample surface. The signal processing problems include the decomposition of a force spectrum into elementary patterns and the factorization of a force-volume image. We discuss the ability of decomposition methods to solve these problems and we illustrate the discussion by means of experimental data.

Index Terms— Atomic force microscopy (AFM), force-volume imaging, tridimensional signals, convolutive mixture of signals.

1. INTRODUCTION

Interatomic and intermolecular forces have been extensively studied for their ability to understand the processes at the interface between solids and aqueous solutions. During the last decades, the development of near field microscopies has afforded to determine *in situ* local physico-chemical properties (electric, magnetic, vibration, forces) [1]. In particular, atomic force microscopy (AFM) is capable of generating force profiles at nanometric scale whatever the nature of the samples (biological, organic, mineral), and tridimensional (3D) images called *force-volume images* [2].

An AFM microscope is based on the measurement of interatomic forces exerting between a probe associated to a cantilever and a sample. A force spectrum $f(z)$ shows the force evolution as a function of the probe-sample distance z as recorded from the piezo displacement. This is a pointwise analysis of the sample obtained by measuring the cantilever deflection with respect to (w.r.t.) the probe-sample distance. A reproduction of this analysis in conjunction with the scan of the sample surface yields a force-volume image $f(x, y, z)$ formed of the collection of force spectra $f(z)$ on a grid (x, y) representing the sample surface.

The analysis of a force-volume image remains mainly descriptive and there is a great need for advanced signal processing tools. Such tools will be dedicated to the reconstruction of maps representing the topography of nano-objects and their physico-chemical properties. The resolution of the topographical reconstruction problem will lead to major advances in the interpretation of data generated by other near field microscopy techniques (namely, optical

techniques). Another open problem is the research of elementary physico-chemical components inside a force-volume image. When the sample to be analyzed is a mixture of homogeneous components, the objective is to determine their number, to identify them and to estimate their proportion in the mixture by source separation techniques. The development of multilinear factorization methods opens new prospects since they aim at decomposing multidimensional signals by means of reduced dimension descriptors [3]. We expect to retrieve elementary force interactions from force-volume images and to provide their spatial distribution and their evolution as a function of physico-chemical conditions such as pH or ionic strength.

In the following section, we describe the acquisition of spectroscopic data using the AFM instrument. We emphasize the physical interactions occurring between the probe and the sample and we illustrate the discussion with a set of real data corresponding to mineral colloidal particles whose chemical surface properties are heterogeneous and far to be understood. In Section 3, we introduce the analysis of force spectra and force-volume images in terms of the decomposition of a signal into elementary patterns and the factorization of a multidimensional signal. We discuss the ability of decomposition methods to solve these problems.

2. AFM MICROSCOPY

The operating modes of an AFM microscope are based on the detection of interatomic forces (capillary, electrostatic, Van der Waals, friction) exerting between a cantilever-mounted probe and the sample surface. In the contact and intermittent imaging modes, the probe scans the whole sample surface and provides two-dimensional data. In the contact mode, the probe and the sample remain in close contact during the raster scan yielding the sample topography. In the intermittent mode, isoforce images are recorded as the force acting on the cantilever is kept constant.

2.1. Force spectroscopy

Contrarily to the previous modes, force spectroscopy is a *pointwise* analysis of the sample obtained by measuring the cantilever deflection as a function of the distance z between the probe and the sample surface. A force spectrum $f(z)$ shows the force evolution as a function of z at a specific location on the sample. The general shape of a force spectrum is shown on Fig. 1. The force intensity is computed from relative measurements of the cantilever deflection as a function of the relative motion of the probe z, where the largest z-value stands for the reference probe position whose location is the most distant from the sample. A force profile is composed of two curves corresponding to the approach and the retraction of the probe. We now describe the specific regions of interest on these curves.
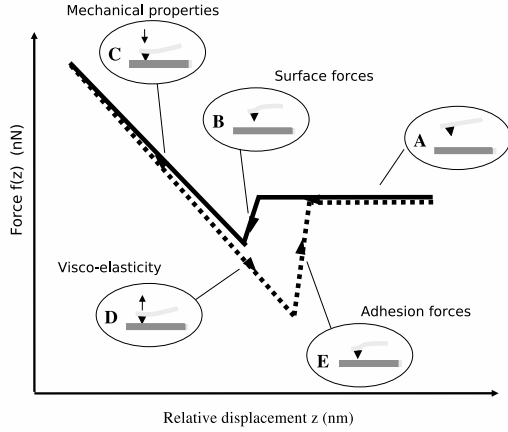
Fig. 1. Approach (solid line) and retraction (dashed line) curves. Adapted from [4].

Approach curve. In region A, no interaction occurs as the cantilever is far from the sample. This region allows us to define the zero value of the forces. Here, let us stress that the experimental force spectra are measured in a relative fashion in terms of both probe motion and force values. The interactions encountered in region B are related to surface forces (electrostatic, Van der Waals). Their sign is either negative (adhesion between the probe and the surface) or positive (repulsion). The contact between the probe and the sample is reached between regions B and C, and region C describes the mechanical interactions of the cantilever and/or the sample. For a non deformable sample, this behavior is mainly due to the linear deformation of the cantilever. For a deformable sample, compression and/or indentation processes lead to linear or non linear behaviors.

Retraction curve. During the retraction, the occurrence of an hysteresis between the approach and retraction curves is due to the viscoelastic properties of the sample (region D). For non deformable surfaces, this hysteresis is equal to zero. In region E, important adhesion forces may be embedded in the retraction curve depending on the surface of contact, the contact duration, and mainly on the surface energy between the sample and the probe. For micro-organisms, this region is composed of numerous discontinuities.

2.2. Force-volume imaging

By reproducing the preceding pointwise analysis and by scanning the sample surface, we obtain a force-volume image $f(x, y, z)$[1]. This image is formed of the collection of force spectra $f(z)$ on a grid (x, y) representing the sample surface (see Fig. 2).

The visualization of such 3D image is not obvious. Typically, one considers each force spectrum separately and then estimates the contact point between the probe and the sample. From these results, it is possible to form a 2D topographical reconstruction of the sample. We now illustrate the AFM modality with a set of real data obtained in aqueous solution using an MFP-3D instrument (Asylum Research, Santa Barbara, USA).

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[1]The recorded signals are discrete but we rather, for simplicity reasons, use continuous notations for x, y, z except when the discrete form is necessary.
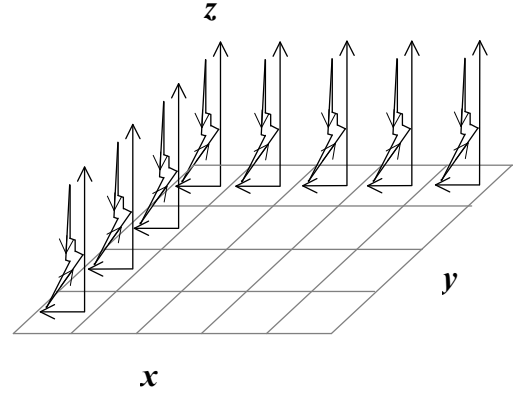

Fig. 2. Recording of a force-volume image on a grid (x, y) representing the sample surface.

2.3. Experimental data

Fig. 3 (a) displays two force spectra measured for a non-deformable nanometric goethite particle (α-FeOOH) immobilized on a glass slide [5]. The exhibited force profiles are related to two locations which belong to the goethite particle surface and the glass slide, respectively (see Fig. 3 (b)). The surface forces are adhesive on the glass slide and repulsive on the goethite particle whose polarization is identical to that of the probe. Moreover, the force profiles show that the topography is different at these two points as the probe-sample contact (regions B-C) is not reached for the same z-value.

The force-volume image which was measured during the AFM experiment corresponds to the scan of a surface $\{(x, y) \subset [x_{\min}, x_{\max}] \times [y_{\min}, y_{\max}]\}$ of size 1 μm^2. The sample surface was discretized into 10×10 square pixels (x_i, y_i) giving rise to the measurement of 100 force spectra $f(x_i, y_i, z)$.

2.4. Signal processing issues

Let us consider the three experimental force spectra shown on Fig. 3 (c). The blue spectrum is related to a nano-particle pixel since the force interaction is repulsive. Similarly, the black spectrum is related to a glass slide pixel. The red spectrum is composed of both adhesion and repulsion features, because the corresponding pixel lays at the border between the nano-particle and the glass slide. In the following, we will distinguish *homogeneous* and *heterogeneous* interactions depending whether the sample is a mixture of several elementary components or not at pixel (x_i, y_i) (see also Fig. 4). In the latter case, the force spectrum will be modeled as a combination of homogeneous elementary spectra.

When a number of pixels (x_i, y_i) describing the sample surface are heterogeneous, the retrieval of elementary components from the mixture can be seen as a source separation problem. We will focus on this problem in the next section. For clarity reasons, we will consider a set of 3D signals $f(x, y, z)$ (*e.g.,* approach curves only), although in practice, the data are formed of two such sets corresponding to approach and retraction curves.

3. ANALYSIS OF A FORCE-VOLUME IMAGE

3.1. Convolutive mixture model

The force-volume image analysis aims at characterizing each pixel (x_i, y_i) of the sample surface given the entire 3D data $f(x, y, z)$.
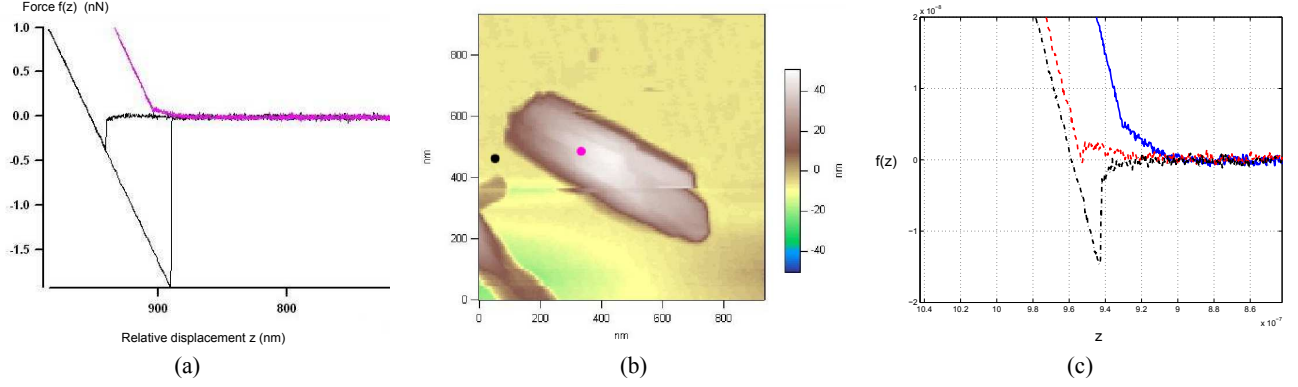
Fig. 3. Force-volume imaging of a goethite nano-particle (α-FeOOH) lain on a glass slide, in interaction with an AFM probe covered with an aluminum oxide of positive charge (pH $= 4$, NaNO$_3 = 1$ mM). (a) Recording of two force spectra in the interior (repulsive interactions) and exterior (adhesive interactions) of the goethite particle surface. (b) AFM image measured in contact mode in liquid environment. (c) Zoom in of three experimental force spectra (approach curve only). The blue and black spectra correspond to repulsive and adhesive interactions, respectively. The red spectrum characterizes a heterogeneous interaction; it is composed of both repulsion and adhesion features.
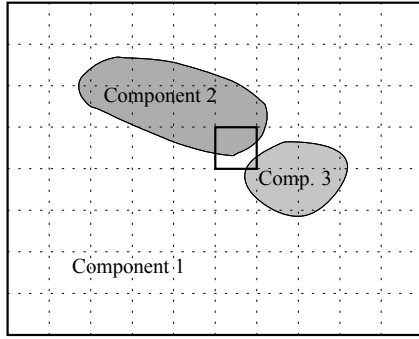

Fig. 4. Discretization of the sample surface into a set of pixels. A pixel is referred to as homogeneous when its surface is composed of only one elementary component (*e.g.*, only component $k = 1$ occurs for the background pixels). The bold pixel is heterogeneous as the result of a mixture of elementary components ($k = 1$, 2 and 3). The force-volume image analysis aims at retrieving the topography and weight of the elementary components into each pixel.

Let us consider a heterogeneous sample composed of p elementary homogeneous components called *sources*. We assume that each data spectrum $f(x_i, y_i, z)$ results from a combination of the source spectra, denoted by $f_1(z), f_2(z), \ldots, f_p(z)$. We use a convolutive mixture model to obtain this description:

$$f(x_i, y_i, z) = \sum_{k=1}^{p} a_{ik}\, f_k(z - z_{ik}) = \sum_{k=1}^{p} a_{ik}\left(\delta_{z_{ik}} \underset{z}{*} f_k\right)(z), \quad (1)$$

where $\delta_{z_{ik}}(z) = \delta(z - z_{ik})$ stands for the 1D Dirac distribution, and z_{ik} is homogeneous to a probe-sample distance. z_{ik} characterizes the topography of the k-th source inside the i-th pixel, and the mixture coefficient a_{ik} is the weight of the k-th source inside the i-th pixel. Assuming that the source supports are distinct and partition the pixel domain, the mixture coefficients satisfy $0 \leqslant a_{ik} \leqslant 1$ and

$$\forall i, \ \sum_{k=1}^{p} a_{ik} = 1. \quad (2)$$

The occurrence of several sources in the i-th pixel indicates that the sample is locally heterogeneous: $a_{ik} \neq 0$ for several values of k. Under the above assumption, heterogeneity only occurs for pixels which lay at the border between several source supports: see Fig. 4.

The joint estimation of the sources, their topography and the mixture coefficients from a force-volume image $f(x, y, z)$ is a source separation problem. It is a classical signal processing problem which however, is far to be trivial for convolutive mixtures.

3.2. Source separation from convolutive mixtures

Clearly, (1) reduces to the classical instantaneous linear model encountered in source separation when the parameters z_{ik} are all equal to zero, *i.e.*, when the sample topography is flat. In general, the topography is unknown (and not flat) as well as the sources and their respective weights in the mixture.

Source separation amounts to estimating the source signals f_k (and their number p), their topography z_{ik} and the mixture coefficients a_{ik} given the experimental data and model (1). It is well known that this problem is ill-conditioned and suffers from several indeterminacies. In particular, the sources f_k can only be separated up to a delay z_k' since

$$\delta_{z_{ik}} * f_k = \left(\delta_{z_{ik}} * \delta_{-z_k'}\right) * \left(\delta_{z_k'} * f_k\right). \quad (3)$$

It is essential to impose constraints to ensure the identifiability of the mixture. Typically, the sources are assumed to be white and independent [6]. Unfortunately, this hypothesis is not compatible with the shape of the homogeneous force spectra. An alternative approach relies on a parsimonious representation of the sources, which can be obtained, *e.g.*, by detecting the discontinuities of the force spectra and their first order derivatives. Here, we rather introduce constraints based on the physical models describing the probe-sample interaction in the approach (van der Waals, electrostatic, elastic forces) and retraction (adhesion and chemical bonding) phases. For each type of interaction, we resort to a parametric model of the force spectrum.

3.3. Use of parametric models of force spectra

We express the source signals f_k in a parametric fashion, where the parameters $\boldsymbol{\theta}_k$ refer to a set of elementary patterns (discontinuity,

exponential curve, *etc.*) embedded in the force spectra:

$$f_k(z; \boldsymbol{\theta}_k) = \sum_{l=1}^{L} b_{kl}\, g_l(z; \boldsymbol{\theta}_{kl}). \tag{4}$$

In this decomposition, $\boldsymbol{\theta}_{kl}$ is the shape factor of the l-th elementary pattern g_l in the k-th source. If a given pattern is absent from a source signal, the number of terms occurring in (4) is reduced by setting b_{kl} to zero. Finally, the mixture model (1) rereads

$$f(x_i, y_i, z) = \sum_{k=1}^{p} \sum_{l=1}^{L} a_{ik}\, b_{kl}\, g_l(z - z_{ik}; \boldsymbol{\theta}_{kl}). \tag{5}$$

For the goethite data illustrated on Fig. 3, there are two sources corresponding to the nano-object and the background, *i.e.,* the glass slide, respectively. In the approach phase, the elementary patterns are the exponential and affine curves embedded in the homogeneous spectra (see Fig. 3 (a)). The first source related to the nano-object alone (repulsive interaction) reads:

$$f_1(z; \boldsymbol{\theta}_1) = \begin{cases} \tau \exp(z/\tau) & \text{if } z \leqslant 0, \\ \tau + az & \text{if } z > 0, \end{cases} \tag{6}$$

with $\boldsymbol{\theta}_1 = \{a, \tau\}$ and $a, \tau \geqslant 0$. This model is also valid for adhesive interactions, where τ is now negative while a is actually common to both models as it is an intrinsic characteristic of the AFM probe.

Future work will be dedicated to the influence of the decomposition (5) on the identifiability of the convolutive mixture. We will carry out the source separation by jointly estimating the parameters a_{ik}, z_{ik}, b_{kl} and $\boldsymbol{\theta}_{kl}$. We expect that the modeling of prior information (a_{ik} and z_{ik} have smooth spatial variations, and a_{ik} is binary if the i-th pixel does not lay at the border between several source supports) will afford us to solve the source separation problem.

3.4. Influence of the probe geometry

The mixture model (1) is often unrealistic as it does not take the probe geometry into account. It is valid when the probe width is negligible w.r.t. the size of the pixels discretizing the sample surface, or equivalently, when the sample surface is smooth enough in comparison with the probe width. When this condition is not satisfied, one needs a more realistic model which also involves a convolution in the (x, y) domain [7].

Let us assume that the probe extremity is non deformable, flat and of parallelepipedic shape. Then each pixel of the sample surface is submitted to a force distribution related to the probe geometry. We thus generalize the mixture model as follows:

$$f(x, y, z) = h(x, y) \underset{(x,y)}{*} \sum_{k=1}^{p} \Big[a_{xyk}\, f_k(z - z_{xyk}) \Big], \tag{7}$$

where x_i, y_i, a_{ik} and z_{ik} are replaced by continuous notations. The point spread function h is defined by:

$$h(x, y) = \begin{cases} 1 & \text{if } (x, y) \in [x_{\min}, x_{\max}] \times [y_{\min}, y_{\max}], \\ 0 & \text{otherwise}, \end{cases} \tag{8}$$

and $\big[x_{\min}, x_{\max}\big] \times \big[y_{\min}, y_{\max}\big]$ stands for the horizontal cross-section of the probe. Model (7) can be generalized for non flat probes. The point spread function h would not only depend on (x, y), but also on z to take account of the relative depth of the probe extremity.

When the force-volume data are finely resoluted along the (x, y) dimension, we expect to reconstruct $2p$ fine resoluted images representing the sample topography z_{xyk} and the weights a_{xyk} of the elementary components. The joint estimation of $h(x, y)$ and the mixture parameters will then be expressed as a deconvolution problem coupled with a source separation problem. A natural strategy to cope with it is to identify h and then to remove the probe geometry influence prior to the resolution of the source separation problem. Indeed, the identification step can be done by means of a learning sequence corresponding to a flat mineral nano-object lying on a glass slide.

4. CONCLUSIONS

We have introduced the AFM modality and the physical processes related to the record of force-volume images. Firstly, we have introduced a convolutive mixture model describing heterogeneous interactions by means of homogeneous elementary components. The convolution operator takes account of the topography of the elementary components and the probe geometry. Secondly, we have exhibited parametric models describing an elementary component alone by a collection of shape factors, *e.g.,* discontinuities or exponential curves. We have illustrated these models with a set of experimental data obtained in aqueous solution. This methodology can be applied to more complex biological systems, like the microbial cells studied in [4]. From a signal processing standpoint, future works will aim at developing advanced methods dedicated to force-volume images, namely the factorization and the deconvolution of a force-volume image. We expect that the use of multilinear tensor factorization algorithms coupled with the parametric description of elementary spectra will afford us to solve the source separation problem.

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